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7. Biochemistry of Cancer

Raymond W. Ruddon MD PhD

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Historical Perspectives

Much of what we originally knew about the biochemical differences between normal and malignant cells was discovered in their patterns of enzymatic activity. In the 1920s, Warburg studied glycolysis in a wide variety of human and animal tumors and found that there was a general trend toward an increased rate of glycolysis in tumor cells.[1] He noted that when normal tissue slices were incubated in a nutrient medium containing glucose, but without oxygen, there was a high rate of lactic acid production (anaerobic glycolysis); however, if they were incubated with oxygen, lactic acid production virtually stopped. The rate of lactic acid production was higher in tumor tissue slices in the absence of oxygen than in normal tissues, and the presence of oxygen slowed, but did not eliminate, lactic acid formation in the tumor slices. Warburg concluded that cancer cells have an irreversible injury to their respiratory mechanism, which increases the rate of lactic acid production even in the presence of oxygen. He regarded the persistence of this type of glycolysis as the crucial biochemical lesion in neoplastic transformation. This old idea still has some credence in that there are hypoxic areas in the core of tumors, where anaerobic metabolism predominates. This has clinical implications because hypoxic cells do not respond as well to certain anticancer drugs and radiation therapy. Interestingly, although it was originally thought that these hypoxic areas were only in the center of tumors and remained relatively static and eventually became necrotic, it is now known that hypoxic areas actually come and go in a tumor as perfusion varies and as new blood vessels form, fade away, and then reform.[2] Certain drugs and radiosensitizers have been designed to take advantage of these hypoxic areas.

The mechanism for this ability of cancer cells to undergo aerobic glycolysis appears to involve the c-*myc* oncogenic transcription factor. Dang et al[3] have shown that c-*myc* and the hypoxia-inducible factor 1 (HIF-1) are able to bind the lactate dehydrogenase A promoter cis-acting elements. They hypothesize that c-*myc* directly activates hypoxia/glucose-responsive elements in glycolytic enzyme genes to increase the ability of cancer cells to maintain aerobic glycolysis.

In the early 1950s, Greenstein formulated the "convergence hypothesis" of cancer, which states that the enzymatic activity of malignant neoplasms tends to converge to a common pattern.[4] Although he recognized some exceptions

to this rule, he considered the generalization, based mostly on repeatedly transplanted tumor models, to be valid. It is now more fully appreciated that even though cancer cells do have some commonly increased metabolic pathways, such as those involved in nucleic acid synthesis, there is tremendous biochemical heterogeneity among malignant neoplasms, and that there are many fairly well-differentiated cancers that do not have the common enzymatic alterations he suggested. Thus, cancers do not have a universally uniform malignant phenotype as exemplified by their enzyme patterns.

On the basis of work of about 50 years ago, which evolved from studies on the production of hepatic cancer by feeding aminoazo dyes, the Millers advanced the "deletion hypothesis" of cancer.[5] This hypothesis was based on the observation that a carcinogenic aminoazo dye covalently bound liver proteins in animals undergoing carcinogenesis, whereas little or no dye binding occurred with the protein of tumors induced by the dye. They suggested that carcinogenesis resulted from "a permanent alteration or loss of protein essential for the control of growth."

About 10 years later, Potter suggested that the proteins lost during carcinogenesis may be involved in the feedback control of enzyme systems required for cell division,[6] and he proposed the "feedback deletion hypothesis."[7] In this hypothesis, Potter postulated that "repressors" crucial to the regulation of genes involved in cell proliferation are lost or inactivated by the action of oncogenic agents on the cell, either by interacting with DNA to block repressor gene transcription or by reacting directly with repressor proteins and inactivating them. This prediction anticipated the discovery of tumor suppressor proteins, such as p53 and RB by, about 25 years.

Biochemical studies of cancer were also aided by the so-called minimal-deviation hepatomas developed by Morris and his colleagues.[8] These tumors were originally induced in rats by feeding them the carcinogens fluorenylphthalamic acid, fluorenylacetamide compounds, or trimethylaniline. These hepatocellular carcinomas are transplantable in an inbred host strain of rats and have a variety of growth rates and degrees of differentiation. They range from slowly growing, well-differentiated, karyotypically normal cells to rapidly growing, poorly differentiated, polyploid cells. All these tumors are malignant and eventually kill the host. The term "minimal deviation" was coined by Potter[7] to convey the idea that some of these neoplasms differ only slightly from normal hepatic parenchymal cells. The hypothesis was that if the biochemical lesions present in the most minimally deviated neoplasm could be identified, the crucial changes defining the malignant phenotype could be determined. As Weinhouse[9] has indicated, studies of these tumors greatly advanced our knowledge of the biochemical characteristics of the malignant phenotype, and they have ruled out many secondary or nonspecific changes that relate more to tissue growth rate than to malignancy.

The extensive biochemical analyses of the Morris minimal-deviation hepatomas led Weber to formulate the "molecular correlation concept" of

cancer, which states that "the biochemical strategy of the genome in neoplasia could be identified by elucidation of the pattern of gene expression as revealed in the activity, concentration, and isozyme aspects of key enzymes and their linking with neoplastic transformation and progression."[10] Weber proposed three general types of biochemical alterations associated with malignancy: (1) transformation-linked alterations that correlate with the events of malignant transformation and that are probably altered in the same direction in all malignant cells; (2) progression-linked alterations that correlate with tumor growth rate, invasiveness, and metastatic potential; and (3) coincidental alterations that are secondary events and do not correlate strictly with transformation or progression. Weber maintained that key enzymes, that is, enzymes involved in the regulation of rate and direction of flux of competing synthetic and catabolic pathways, would be the enzymes most likely to be altered in the malignant process. In contrast, "nonkey" enzymes, that is, enzymes that are not rate limiting and do not regulate reversible equilibrium reactions, would be of lesser importance. As one would expect, a number of enzyme activities that Weber and others have found to be altered in malignant cells are those involved in nucleic acid synthesis and catabolism. In general, the key enzymes in the de novo and salvage pathways of purine and pyrimidine biosynthesis are increased and the opposing catabolic enzymes are decreased during malignant transformation and tumor progression. Weber noted that the degree of neoplasia was related to the concentrations of certain regulators of key metabolic pathways. Those metabolic pathways that contained enzymes which fulfilled one or more of these criteria are indicated in Table 7.1 along with the alteration that was observed in cancer. The question of why anaplastic, rapidly growing tumors tend to be biochemically alike, whereas more well-differentiated tumors display a vast array of phenotypic characteristics, was approached by Knox.[11] He thought that the vast bulk of biochemical components in tumor tissues are "normal," in the sense that they are produced by certain specialized adult normal cells or by normal cells at some stage of their differentiation. In cancer cells, it is the combination and proportions of these normal components that are abnormal. The biochemical diversity of cancer cells, then, would depend on the cell of origin of the neoplasm and its degree of neoplasticity.[11] All too frequently, even now, in the histopathologic or biochemical characterization of cancer, a biochemical component that is present or absent or increased or decreased is not considered in relation to the particular cell of origin of a tumor, its differentiation state, or its degree of neoplasticity.

Taken together, the data on enzyme patterns of cancer cells indicate that undifferentiated, highly malignant cells tend to resemble one another and fetal tissues more than their adult normal counterpart cells, whereas well-differentiated tumors tend to resemble their cell of origin more than other tumors. Of course, between these two extremes several levels of neoplastic gradation occur, leading to the vast biochemical heterogeneity of tumors. This heterogeneity also exists for tumors of the same tissue type arising in different patients or even in the same patient at different stages of the disease.

The fact that more undifferentiated tumors tend to converge to a more fetal-

like state is evidenced by a frequently observed production of oncodevelopmental gene products. A number of cancer cell characteristics, such as invasiveness and "metastasis," are also seen in embryonic tissue. For example, the developing trophoblast invades the uterine wall during the implantation step of embryonic development. During organogenesis, embryonic cells dissociate themselves from the surrounding cells and migrate to new locations, a process not unlike metastasis. All these, however, are controlled processes with a definitive end point, whereas in cancer, similar events occur but in a dysregulated manner. One of the keys to understanding neoplasia may be to discover what regulates the expression of these embryonic processes. Which, if any, of these gene products are involved in cancer's invasion, metastatic spread, and avoidance of host immunologic defense mechanisms is not entirely clear, but it is likely that at least some of them are involved; for example, proteases that act, in a controlled manner during blastocyst implantation, function in a dysregulated way in invasive cancer. What regulates the invasive and metastatic properties of malignant cells is still a crucial question in cancer biology.

A number of oncodevelopmental tumor-associated antigens appear on tumor cells as a result of the apparent re-expression (or increased expression) of embryonic genes, and a number of these are useful as tumor markers for cancer diagnosis and disease progression. These include alpha-fetoprotein (AFP), carcinoembryonic antigen (CEA), and a number of inappropriately (ectopically) produced hormones (Table 7.2).

The production of the placental hormone human chorionic gonadotropin (hCG) or of its alpha or beta subunits, for example, has been observed in a number of human cancer cell lines[12] and in the serum of patients with ovarian, urinary bladder, or gastrointestinal cancers.[13–15] It appears that hCG or its unassembled beta subunit (hCG normally is present as an α-β heterodimer in normal pregnancy serum) is a growth factor for certain cancers, and a therapeutic approach based on this has been developed, using an antibody to hCG.[16]

A number of chromosomal abnormalities were observed in cancer cells many years ago. In 1914, Boveri[17], formulated the somatic mutation hypothesis of the origin of cancer. He thought that the origin of the cancer cell was due to a "wrongly combined chromosome complex," occurring in a somatic cell (rather than a germ cell), and that this caused abnormal cell proliferation. He believed further that this defect was passed on to all cellular descendants of the original cancer cell. He also thought that a single abnormal chromosome combination could account for the malignant character of a cancer cell. It is now well established that certain chromosomal rearrangements are associated with human malignant neoplasia, and that some human cancers have a familial distribution.

Modern molecular biology and biotechnology have made the detection of genetic abnormalities in cancer cells a more precise and complete science. For example, the use of the polymerase chain reaction (PCR) has enabled

investigators to amplify a tiny sample of DNA or RNA extracted from a tumor a million-fold or more in order to detect subtle genetic alterations, such as single base changes that may be involved in activation of an oncogene or mutation of a tumor suppressor gene.[18] The employment of DNA microarrays has provided a means to examine the expression of thousands of genes on a single DNA chip.[19] Laser capture micro-dissection techniques can release a single cell from a tissue sample for analysis by PCR and DNA microarray to, in effect, provide a snapshot of the entire genomic expression of a single cell.[20] TOP

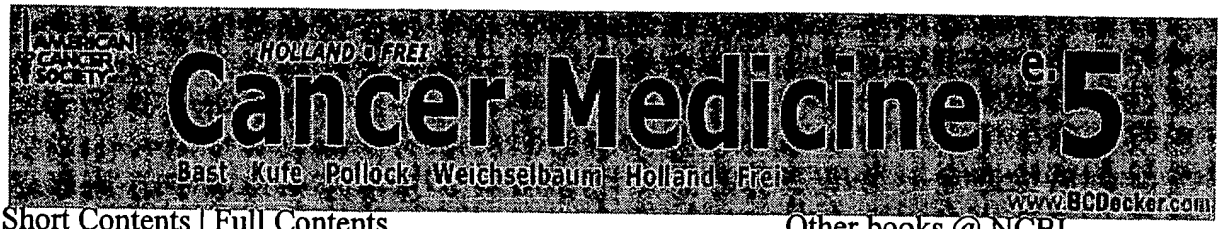

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Table 7.1. Molecular Correlation Concept and Affected Processes

Biochemical Process	Alteration in Cancer Cells
Pyrimidine and purine synthesis	Increased
Pyrimidine and purine catabolism	Decreased
RNA and DNA synthesis	Increased
Glucose catabolism	Increased
Glucose synthesis	Decreased
Amino acid catabolism (for gluconeogenesis)	Decreased
Urea cycle	Decreased

Adapted with permission from Weber.[9]

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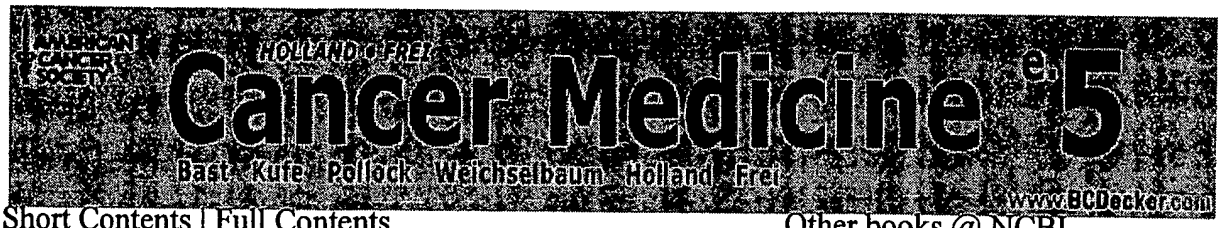
Table 7.2. Ectopic Hormones Produced by Various Human Cancers

Hormone Activity	Tumors Producing Hormone Ectopically	Associated Clinical Syndrome
ACTH	Carcinomas of lung, colon, pancreas, thyroid, prostate, ovary, cervix; thymoma; pheochromocytoma; carcinoid tumors	Cushings' syndrome
ADH	Carcinomas of lung, duodenum, pancreas, uterus, prostate; thymoma; lymphoma; Ewing's sarcoma	Inappropriate antidiuresis, hyponatremia
Calcitonin	Carcinomas of lung, breast, prostate, bladder, pancreas, liver, esophagus, stomach, colon, larynx, testis; carcinoid tumors; insulinoma; pheochromocytoma; melanoma	No apparent syndrome
Erythropoietin	Hemangioblastoma; uterine myofibroma; pheochromocytoma; carcinoma of liver, ovary	Polycythemia (erthrocytosis)
Gastrin	Carcinoma of pancreas	Zollinger-Ellison syndrome (gastric hypersecretion with intractable peptic ulceration)
Glucagon	Carcinoma of kidney	Hyperglycemia , malabsorption, gastrointestinal stasis
Growth hormone	Carcinomas of lung, stomach, ovary, breast	Hypertrophic pulmonary osteoarthropathy, acromegaly
hCG	Carcinomas of breast, stomach,	Gynecomastia,

	small intestine, pancreas, biliary tract, liver, colon, rectum, lung, ovary, testis; melanoma	precocious puberty
Prolactin	Carcinomas of lung, kidney	Galactorrhea, gynecomastia
PTH	Carcinomas of kidney, lung, liver, adrenal, pancreas, parotid, ovary, testis, spleen, breast	Hypercalcemia
TSH	Carcinomas of lung, breast	Hyperthyroidism

ACTH = adenocorticotropic hormone; ADH = antidiuretic hormone; hCG = human chorionic gonadotropin; PTH = parathyroid hormone; TSH = thyroid-stimulating hormone.



What Makes a Cancer Cell a Cancer Cell?

Phenotypic Alterations in Cancer Cells

Treatment of animals or cells in culture with carcinogenic agents is a means of studying discrete biochemical events that lead to malignant transformation. Studies of cell transformation in vitro, however, have many pitfalls. These "tissue culture artifacts" include overgrowth of cells not characteristic of the original population of cultured cells (e.g., overgrowth of fibroblasts in cultures that were originally primarily epithelial cells), selection for a small population of variant cells with continued passage in vitro, or appearance of cells with an abnormal chromosomal number or structure (karyotype). Such changes in the characteristics of cultured cell populations can lead to "spontaneous" transformation that mimics some of the changes seen in populations of cultured cells treated with oncogenic agents. Thus, it is often difficult to sort out the critical malignant events from the noncritical ones. Malignant transformation can also be induced in vivo, by treatment of susceptible experimental animals with carcinogenic chemicals or oncogenic viruses or by irradiation, but identification of critical biochemical changes in vivo is even more tenuous because it is difficult to discriminate toxic from malignant events and to determine what role a myriad factors, such as the nutritional state of the animal, hormone levels, or endogenous infections with microorganisms or parasites, might have on the in vivo carcinogenic events. Moreover, tissues in vivo are a mixture of cell types, and it is difficult to determine in which cells the critical transformation events are occurring and what role the microenvironment of the tissue plays. Most studies that are designed to identify discrete biochemical events occurring in cells during malignant transformation have therefore been done with cultured cells, since clones of relatively homogeneous cell populations can be studied and the cellular environment defined and manipulated. The ultimate criterion that establishes whether or not cells have been transformed, however, is their ability to form a tumor in an appropriate host animal. The recently developed ability to generate immortalized "normal" cell lines of a given differentiated phenotype from human embryonic stem cells has enhanced the ability to study cells of a normal genotype from a single source.[21] Such cell lines may be generated by transfection of the telomerase gene into cells to maintain chromosomal length.

Over the past 60 years, much scientific effort has gone into research aimed at

identifying the phenotypic characteristics of in vitro–transformed cells that correlate with the growth of a cancer in vivo. This research has tremendously increased our knowledge of the biochemistry of cancer cells. However, many of the biochemical characteristics initially thought to be closely associated with the malignant phenotype of cells in culture have subsequently been found to be dissociable from the ability of those cells to produce tumors in animals. Furthermore, individual cells of malignant tumors growing in animals or in humans exhibit marked biochemical heterogeneity, as reflected in their cell surface composition, enzyme levels, immunogenicity, response to anticancer drugs, and so on. This has made it extremely difficult to identify the essential changes that produce the malignant phenotype. Recently, however, Hahn et al.[22] have shown that ectopic expression of the human telomerase catalytic subunit (hTERT) in combination with the oncogenes h-*ras* and SV40 virus large-T antigen can induce tumorigenic conversion in normal human epithelial and fibroblast cells, suggesting that disruption of the intracellular pathways regulated by these gene products is sufficient to produce a malignant cell. Table 7.3 lists the properties of transformed malignant cells growing in cell culture or in vivo.[23]

Some of these characteristics may be seen both in transformed cells in culture and in tumors growing in vivo in experimental animals or patients. Some of the characteristics listed in Table 7.3 may also be observed in rapidly proliferating tissues or stem cell populations of undifferentiated phenotypes. In addition, hyperproliferative conditions in patients, such as inflammatory bowel disease or psoriasis, may have some of these characteristics. Thus, for diagnostic purposes, it is important to use a number of characteristics that define the malignant state. The evidence that these phenotypic properties found in transformed cells are related to malignant neoplasia is discussed below.

Immortality of Transformed Cells in Culture.

Most normal diploid mammalian cells have a limited life expectancy in culture. For example, normal human fibroblast lines may live for 50 to 60 population doublings (the "Hayflick index"), but then viability begins to decrease rapidly unless they transform spontaneously or are transformed by oncogenic agents. However, malignant cells, once they become established in culture, will generally live for an indefinite number of population doublings, provided the right nutrients and growth factors are present. It is not clear what limits the life expectancy of normal diploid cells in culture, but it may be related to the continual shortening of chromosomal telomeres each time cells divide. Transformed cells are known to have elevated levels of telomerase that maintain telomere length. Transformed cells that become established in culture also frequently undergo karyotypic changes, usually marked by an increase in chromosomes (polyploidy), with continual passage. This suggests that cells with increased amounts of certain growth-promoting genes are generated and/or selected during continual passage in culture. The more undifferentiated cells from cancers of animals or patients also often have an atypical karyology, suggesting that the same selection process may be going on in vivo with progression over time of malignancy from a lower

to a higher grade. TOP

Decreased Density-Dependent Inhibition of Growth.

It has been known for a long time that "the epithelium will not tolerate a free edge,"[24] that is, with a suitable surface to grow on, a "free edge" of epithelial cells will replicate and move until it makes contact with another edge of epithelial cells. For example, if the skin of an amphibian larva is wounded, a rim of epidermis migrates out from the wound until the free edges of this expanding rim meet in the middle of the wound, whereupon epithelial extension abruptly ceases.[25] Fibroblasts have been shown to have a similar sort of contact reaction, as exemplified by chick-heart fibroblasts moving in liquid medium on a glass surface.[26] When two primary explants of these cells were placed 1 mm apart, an outgrowth toward each explant occurred until the cells made contact. At that point, outgrowth and movement of the cells stopped and the cells formed a monolayer on the surface. It was concluded that the cells inhibit one another by mutual contact of their cell surfaces, a phenomenon called *contact inhibition.*[26] A variety of malignant transformed cells do not stop replicating when they come into contact. There is often a good correlation between the ability of transformed mouse fibroblasts to continue to divide in the presence of extensive cell-cell contact in culture and their capability of producing tumors in animals. TOP

Decreased Requirement for Growth Factors.

Another property that distinguishes transformed cells from their nontransformed counterparts is the requirement for growth factors for replication in culture. Cells transformed by oncogenic viruses have lower serum growth requirements than do normal cells.[27] For example, 3T3 fibroblasts transformed by SV40,[28] polyoma,[29] murine sarcoma virus,[29] or Rous sarcoma virus[30] are all able to grow in a culture medium that lacks certain serum growth factors, whereas uninfected cells are not. TOP

Loss of Anchorage Dependence.

Most freshly isolated normal animal cells and cells from cultures of normal diploid cells do not grow well when they are suspended in fluid or a semisolid agar gel. If these cells make contact with a suitable surface, however, they attach, spread, and proliferate. This type of growth is called *anchorage-dependent growth.* Many cell lines derived from tumors and cells transformed by oncogenic agents are able to proliferate in suspension cultures or in a semisolid medium (methylcellulose or agarose) without attachment to a surface. This is called *anchorage-independent growth.* This property of transformed cells has been used to develop clones of malignant cells.[31] This technique has been widely used to compare the growth properties of normal and malignant cells. Another advantage that has been derived from the ability of malignant cells to grow in soft agar (agarose) is the ability to grow cancer cells derived from human tumors to test their sensitivity to chemotherapeutic

agents and to screen for potential new anticancer drugs.[32]

The ability of malignant cells to proliferate autonomously in culture without the addition of serum or hematopoietic growth factors (cytokines) can also have prognostic significance. For example, in a study of 114 patients with acute myelogenous leukemia (AML), a high correlation was found between the ability of a given patient's AML cells to proliferate autonomously in culture and disease prognosis.[33] Those patients whose cells had high rates of autonomous proliferation had a poorer response to chemotherapy, a reduced probability of complete remission, and a higher frequency of relapse than those patients whose AML cells had low in vitro proliferative capacity. The ability of AML cells to proliferate autonomously in culture appears to relate to the cells' ability to produce their own cytokines (e.g., granulocyte macrophage colony-stimulating factor [GM-CSF], G-CSF, M-CSF, interleukin-1 [IL-1], and interleukin-6 [IL-6]). TOP

Loss of Cell Cycle Control and Resistance to Apoptosis.

Normal cells respond to a variety of suboptimal growth conditions by entering a quiescent phase in the cell division cycle, the G0 state. There appears to be a decision point in the G1-phase of the cell cycle, at which time the cell must make a commitment to continue into the S-phase, the DNA synthesis step, or to stop in G1 and wait until conditions are more optimal for cell replication to occur. If this waiting period is prolonged, the cells are said to be in a G0-phase. Once cells make a commitment to divide, they must continue through S, G2, and M to return to G1. If the cells are blocked in S, G2, or M for any length of time, they die. The events that regulate the cell cycle, called *cell cycle check points*, are defined in more detail below. This loss of cell cycle check point control by cancer cells may contribute to their increased susceptibility to anticancer drugs. Normal cells have mechanisms to protect themselves from exposure to growth-limiting conditions or toxic agents by calling on these check-point control mechanisms. Cancer cells, on the other hand, can continue through these check points into cell cycle phases that make them more susceptible to the cytotoxic effects of drugs or irradiation. For example, if normal cells accrue DNA damage due to ultraviolet (UV) or x-irradiation, they arrest in G1 so that the damaged DNA can be repaired prior to DNA replication. Another check point in the G2-phase allows repair of chromosome breaks before chromosomes are segregated at mitosis. Cancer cells, which exhibit poor or absent check-point controls, proceed to replicate the damaged DNA, thus accounting for persisting and accumulating mutations. TOP

Changes in Cell Membrane Structure and Function

The cell surface membrane (plasma membrane) plays an important role in the "social" behavior of cells, that is, communication with other cells, cell movement and migration, adherence to other cells or structures, access to nutrients in the microenvironment, and recognition by the body's immune system. Alterations of the plasma membrane in malignant cells may be inferred from a variety of properties that characterize their growth and

behavior, for example, the loss of density-dependent inhibition of growth, decreased adhesiveness, loss of anchorage dependence, and invasiveness through normal tissue barriers. In addition, a number of changes in the biochemical characteristics of malignant cells' surfaces have been observed. These include appearance of new surface antigens, proteoglycans, glycolipids, and mucins, and altered cell-cell and cell–extracellular matrix communication.

Agglutinability of Transformed Cells.

One of the characteristics that defines the plasma membranes of transformed cells is their increased agglutination by such lectins as wheat germ agglutinin (WGA), concanavalin A (Con A), and phytohemagglutinin (PHA). Lectins, from the Latin term *lectus*, meaning "to select," are usually glycoproteins extracted from plants, but they have also been found in bacteria, fungi, fish, snails, and mammals, including human cells. They bind specifically to certain carbohydrates.[34] Thus, they have been used as probes to determine cell agglutinability as well as the glycoprotein and glycolipid composition and the configuration of the cell surface membranes of normal and transformed cells.

Lectin binding by cancer cells has also been used to distinguish different malignant characteristics, including metastatic potential. For example, the most consistent change in cell surface oligosaccharide expression distinguishing high-metastatic-potential from low-metastatic-potential murine lymphoma cells was the ability of the high metastatic potential cells to bind soybean agglutinin lectin.[35] Since this lectin binds N-acetyl-D-galactosamine residues, it suggests that oligosaccharides bearing this residue are more prevalent or more available for binding on cells of high metastatic potential. TOP

Alterations in Cell Surface Glycolipids, Glycoproteins, Proteoglycans, and Mucins.

Aberrant glycosylation was first suggested as the basis for the tumor-associated determinants of glycolipids by the finding of a remarkable accumulation of fucose-containing glycolipids found in human adenocarcinomas, some of which were identified as lactofucopentaose-III-ceramide, lactofucopentaose-II-ceramide (Lewis A blood group glycolipid), and lactodifucohexose and lactodifucooctose ceramide (Lewis B glycolipid).[36] These identifications were confirmed once monoclonal antibodies (mAbs) were used to identify antigens definitively. A number of mAbs with preferential reactivity for tumor cells over normal cells have been shown to react with Lewis blood group antigens, such as Le^x, Le^a, Le^b or their analogues.[36]

The biochemical characterization or the aberrant glycosylation of glycoproteins was also demonstrated in earlier studies. The presence of high-molecular-weight glycopeptides with altered glycosylation patterns was detected on transformed cells in early studies before they were clearly

chemically identified.[37,38] Later, the chemical basis for some of the changes in tumor cell glycoproteins was attributed to the fact that the N-linked oligosaccharides of tumor cells contain more multiantennary structures than the oligosaccharides derived from normal cells.[39]

Tumor-associated carbohydrate antigens can be classified into three groups:[36] (1) epitopes expressed on both glycolipids and glycoproteins; (2) epitopes expressed only on glycolipids; and, (3) epitopes expressed only on glycoproteins. To the first group belongs the lacto-series structure that is found in the most common human cancers, such as lung, breast, colorectal, liver, and pancreatic cancers. The common backbone structure for these epitopes is Galβ1 3GlcNAcβ1 3Gal (type 1 blood group) or Galβ1 4 GlcNacβ1 3 Gal (type 2 blood group). The second group of epitopes, expressed exclusively on glycolipids, are mostly on the ganglio- or globo-series structures. This series of epitopes are expressed abundantly only on certain types of human cancers, such as melanoma, neuroblastoma, small cell lung carcinoma, and Burkitt's lymphoma. The third group of epitopes, seen only on glycoproteins, are the multiantennary branches of N-linked carbohydrates and the alterations of O-linked carbohydrate chains seen in some mucins.

Tumor-associated carbohydrate antigens can also be classified by the cell types expressing them as those (1) expressed on only certain types of normal cells (often only in certain developmental stages) and greatly accumulated in tumor cells; (2) expressed only on tumor cells, for example, altered blood group antigens or mucins; and (3) expressed commonly on normal cells but present in much higher concentrations on tumor cells, for example, the GM_3, ganglioside in melanoma and Le^x in gastrointestinal cancer.[36]

A variety of chemical changes in tumor cells have been identified that can explain altered glycosylation patterns. These result from three kinds of altered processes: (1) incomplete synthesis and/or processing of normally existing carbohydrate chains and accumulation of the resulting precursor form; (2) "neosynthesis" resulting from activation of glycosyltransferases that are absent or have low activity in normal cells; and (3) organizational rearrangement of tumor cell membrane glycolipids.[40] Similar changes have been noted in the carbohydrate components of glycolipids and of membrane-associated and secreted glycoproteins.[41]

Interest in the carbohydrate components of cell surface glycolipids, glycoproteins, and proteoglycans has been heightened by the fact that many of the monoclonal antibodies developed to tumor-cell-associated antigens recognize these carbohydrate moieties or peptide epitopes exposed by altered glycosylation. Moreover, many of these have turned out to be blood group–specific antigens or modifications of blood group–specific antigens, some of which are antigens that are seen at certain stages of embryonic development and thus fit the definition of oncodevelopmental antigens. TOP

Role of Glycosyl Transferases and Oligosaccharide Processing Enzymes.

The substitution of additional carbohydrate moieties on blood group–related structures is not the only aberrant modification of glycoproteins or glycolipids observed in cancer cells. Increased branching of asparagine-linked oligosaccharides and incomplete processing of these oligosaccharides have also been noted in certain cell-surface as well as secretory glycoproteins.[41,42] The increased activity of specific N-acetylglucosaminyl transferases in tumor cells appears to be responsible for the appearance of tri- and tetra-antennary structures, whereas the analogous glycoprotein in normal cells is often a biantennary structure. For example, in polyoma virus–transformed baby hamster kidney (BHK) cells, there is an increase in GlcNAcβ1 6Manα1 groups and a decrease in the more typical GlcNAcβ1 4Manα1 groups on membrane glycoproteins as well as an increase in the activity of GlcNAc transferase V, the enzymatic activity that catalyzes this addition.[41] Moreover, the asparagine-linked oligosaccharides of the placental glycoprotein hCG found in the urine of women with choriocarcinoma have an unusual biantennary structure as well as triantennary sugar chains.[42] Since hCG found in the urine of normal pregnant women does not contain triantennary sugar chains, it appears that increased activity of GlcNAc transferases that produce these additional branched structures is also a feature of human cancer cells. Unusually high expression of N-acetylglucosaminyltransferase-IVa has been observed in human choriocarcinoma cell lines and may be the enzymatic basis for the formation of abnormal biantennary sugar chains on hCG.[43] Similarly, the extra fucosylations that appear on membrane glycoproteins and glycolipids have been associated with the induction of an unusual α-fucosyltransferase in chemical carcinogen–induced precancerous rat liver and in the resulting hepatomas.[44] These investigations strongly suggest that the regulation of glycosyltransferase genes is important in malignant transformation.

A number of other differences in glycosyl transferases have been noted between normal and cancer tissues. Human ovarian carcinomas have several-fold higher levels of α1,3-fucosyl- and α1,4-fucosyltransferases, compared with normal ovarian tissue.[45] Sialyltransferase activity levels have been shown to be higher in metastatic human colon carcinoma cell lines, suggesting that increased sialylation of adhesion molecules may favor implantation of tumor cells into distant tissue sites.[46] Four- to 18-fold increases in the enzyme activity that introduces an additional branch into O-linked glycans has been observed in human AML and chronic myelogenous leukemia (CML) cells.[47] Human melanoma cells that express high levels of the gangliosides GM_3 and GD_3 have high levels of the sialyltransferases involved in their synthesis.[48]

All these data strongly support the idea that glycosylation patterns change during transformation of normal cells into malignant ones. Because cell-cell interactions, adhesion to extracellular matrices, regulation of cell proliferation, and recognition by the host's immune system are all profoundly

affected by the composition of the cell surface, the entire social behavior of a cell could be altered by such changes.

Additional evidence for the importance of glycosylation patterns of cell surface glycoproteins and glycolipids in the malignant phenotype comes from the use of glycosylation inhibitors and oligosaccharide-processing inhibitors. For example, tunicamycin, an inhibitor of addition of N-linked glycans to nascent polypeptide chains, castanospermine, an inhibitor of glucosidase, and KI-8110, an inhibitor of sialyltransferase activity, all reduce the number of lung metastases in murine experimental tumor models.[49–51] In addition, swainsonine was shown to reduce the rate of growth of human melanoma xenografts in athymic nude mice,[52] and castanospermine was observed to inhibit the growth of n-*fms* oncogene-transformed rat cells in vivo.[53] These results support the hypothesis that the synthesis of highly branched complex-type oligosaccharides are associated with the malignant phenotype and may provide tumor cells with a growth advantage. TOP

Mucins.

Mucins are a type of highly glycosylated glycoproteins that a variety of secretory epithelial cells produce. They are 50 to 80% carbohydrate by weight and function to lubricate and protect ductal epithelial cells. They contain O-linked glycans (serine- and threonine-linked) of various lengths and structures, depending on the tissue type in which they are produced. They are made in a wide variety of tissues, including the gastrointestinal tract, lung, breast, pancreas, and ovary, and tumors arising in these organs may have an altered glycosylation pattern that distinguishes them from the normal mucins and renders them immunogenic.

A number of the genes for the core proteins have been cloned and bear names such as *muc1* and *muc2*. The core protein of the *muc1* gene has a transmembrane domain and a large extracellular domain made up of tandem repeats of 20 amino acids; variation in the number of these tandem repeats (30–100) is responsible for the polymorphism observed in the core proteins. Total expression of the mucins is increased in many cancers and upregulated in some normal tissues under different physiologic states (e.g., lactating mammary gland).[54] Increased expression of *muc1*- has been observed in most adenocarcinomas of the breast, lung, stomach, pancreas, prostate, and ovary. Although *muc1* encoded mucin has been the most extensively studied, cancer-related alterations in other mucins have been observed. Moreover, it appears that some cells, both normal and cancer, can express more than one mucin. Focal aberrant expression of *muc2* and *muc3* has been frequently observed in a variety of adenocarcinomas.[55] However, in general, mucin genes appear to be independently regulated and their expression is organ and cell-type specific.[55]

There is evidence for host immune recognition of the breast cancer mucin, in that cytotoxic T lymphocytes isolated from breast cancer patients recognize a mucin epitope expressed on the breast cancer cells.[56] The immune-

recognized epitope involves the core protein that appears to be selectively exposed on breast, ovarian, and other carcinomas. It has also been demonstrated that patients can produce antibodies to cancer mucins,[54] and this is the basis for the proposal that glycopeptides, on the basis of the aberrantly processed mucins of cancer cells, may have some utility as tumor vaccines. Clinical trials of mucin-derived vaccines are underway.[57] Some mucin antigens are shed from tumor cells and can be detected in the sera of patients with pancreatic, ovarian, breast, and colon cancers. These include CA19-9, CA125, CA15-3, SPan-1, and DuPan-2 that are currently being used as tumor markers.[58] TOP

Proteoglycans.

The proteoglycans are high-molecular-weight glycoproteins that have a protein core to which are covalently attached large numbers of side chains of sulfated glycosaminoglycans as well as N-linked and/or O-linked oligosaccharides. They are categorized on the basis of their glycosaminoglycans into several types, including heparan sulfate, chondroitin sulfate, dermatan sulfate, and keratan sulfate.[59] The glycosaminoglycans have different repetitive disaccharide units bound to the core protein through a common glycosaminoglycan linkage region:
GlcNAcβ1 3Galβ1 3Galβ1 4Xylβ1-O-Ser. The structure of the sulfated glycopeptides from the carbohydrate-protein linkage region of some of the proteoglycans has been determined.[60]

Proteoglycans interact via their multiple binding domains with many other structural macromolecules, giving them the capacity "to function as a multi-purpose 'glue' in cellular interactions."[61] They bind together extracellular matrix (ECM) components, such as hyaluronic acid, collagen, laminin, and fibronectin; mediate binding of cells to the ECM; act as a reservoir for growth factors; and "present" growth factors to growth factor receptors on cells. The proteoglycans also act as cell adhesion factors by promoting organization of actin filaments in the cell's cytoskeleton. Proteoglycans have been shown to undergo both quantitative and qualitative changes during malignant transformation, and alterations have been reported in breast, colon, and liver carcinomas, in glioma cells, and in transformed murine mammary cells and 3T3 fibroblasts.

Two putative tumor suppressor genes are glycosyl transferases required for the biosynthesis of the proteoglycan heparan sulfate.[62] Mutations of these genes, called *ext1* and *ext2*, have been associated with the development of skeletal dysplasias, and these findings suggest that alterations in the synthesis of heparan sulfate precursor polysaccharide are involved in dysregulation of heparan sulfate production and function in tumor formation. TOP

Modification of Extracellular Matrix Components.

The ECM plays a key role in regulating cellular proliferation and

differentiation. In the case of tumors, it is now clear that development of a blood supply and interaction with the mesenchymal stroma on which tumor cells grow are involved in their growth, invasive properties, and metastatic potential. This supporting stromal structure is continuously remodeled by the interaction between the growing tumor and host mesenchymal cells and vasculature.

The ECM components include collagen, proteoglycans, and glycoproteins, such as fibronectin, laminin, and entactin. The ECM forms the milieu in which tumor cells proliferate and provides a partial barrier to their growth. Basement membranes are a specialized type of ECM. These membranes serve as a support structure for cells, act as a "sieving" mechanism for transport of nutrients, cellular metabolic products, and migratory cells (e.g., lymphocytes), and play a regulatory role in cell proliferation and differentiation.[63] Basement membranes also prevent the free passage of cells across them, but there are mechanisms that permit the passage of inflammatory cells. It is also clear that basement membranes act as regulators of cell attachment, through cellular receptors called integrins (see below). There is also "cross-talk" between epithelial cells and their ECM to create a microenvironment for accurate signal transduction for growth factors and other regulatory molecules. It has been shown, for example, that exogenous reconstituted basement membranes stimulate specific differentiation of a variety of cell types, including mammary cells, hepatocytes, endothelial cells, lung alveolar cells, uterine epithelial cells, Sertoli cells, and Schwann cells.[64]

The basement membrane barrier can be breeched by tumor cells that release a variety of proteases, glycosidases, and collagenases that have the ability to degrade various components of the matrix and thus allow tumor cells to invade through tissue barriers and blood vessel and lymph channel walls. In addition, malignant cells themselves have receptors for and/or can produce certain components of the matrix; this capability enables them to bind to the vascular endothelium and may be involved in their ability to metastasize. Tumor cells may also release polypeptide factors that can modulate the type of proteoglycans produced by host mesenchymal cells. For example, normal fibroblasts have been shown to produce proteoglycans containing an unusual amount of chondroitin sulfate when they are exposed to conditioned growth medium from cultured human colon cancer cells.[65] TOP

Cell-ECM and Cell-Cell Adhesion.

Cells in tissues are attached to one another and to the ECM. Disruption of these adhesion events leads to increased cell motility and potential invasiveness of cells through the ECM. In addition, most cell types require attachment to the ECM for normal growth, differentiation, and function. This attachment is responsible for what was termed "anchorage dependence." Normal cells cut loose from their binding to the ECM undergo apoptosis, whereas tumor cells that are less dependent on this attachment are free to proliferate, wander, and invade tissues.

Cell adhesion to the ECM is mediated by cell surface receptors called *integrins*. Integrins are a family of proteins consisting of $\alpha\beta$ heterodimers that are integral membrane proteins with a specific arginine, glycine, aspartic acid (RGD) amino acid sequence involved in binding to the ECM.[66] Integrins also link the external ECM cytoskeleton to the intracellular actin cytoskeleton, and via this connection a linkage to control of gene expression in the cell nucleus is established. In this way, cell-ECM interactions can control gene read-out involved in cell differentiation and function. Cell-ECM interactions occur via focal adhesions that consist of clusters of ECM-bound integrins, and these, in turn, connect to actin fibrils and the signal transduction machinery inside the cell. These signaling pathways include the focal adhesion kinase (FAK) pathway that participates in the control of anchorage dependence, and growth factor signaling pathways, such as the *ras-raf*-mitogen-activated kinase, protein kinase C, and phosphatidylinositol 3-kinase pathways.[67] Thus, integrins cooperate with growth factors to enhance mitogenic signaling. Alterations in integrin receptor expression have been observed in chemically transformed human cells and in human colon and breast cancer tissue.[68]

Interestingly, the shape that cells assume as they attach to the ECM can determine whether they live or die.[69] Cells that flatten and spread on the ECM to maximize cell surface attachment survive, whereas rounded cells do not, even though they may remain attached to the ECM. Thus, local control of cell geometery can also regulate cell proliferation and viability and is another mechanism by which cell function is determined by the tissue microenvironment. During malignant transformation, loss of this cell shape–dependent regulation could favor cell proliferation over ECM attachment–determined differentiation.

Cell-cell interactions are also important for the normal regulation of cell proliferation and differentiation. These interactions are mediated by a family of molecules called *cell adhesion molecules* (CAMs), which act as both receptors (on one cell) and ligands (for another cell). The expression of CAMs is programmed during development to provide positional and migratory information for cells. A large family of CAMs has been identified. One group of these, called cadherins, comprise a superfamily of Ca^{++}-dependent transmembrane glycoproteins that play an essential role in the initiation and stabilization of cell-cell contacts. Regulation of cadherin-mediated cell-cell adhesion is important in embryonic development and maintenance of normal tissue differentiation.[70,71]

The extracellular domain of various cadherins is responsible for cell-cell homotypic binding (a given cadherin domain for a given cell type), and the conserved cytoplasmic domains interact with cytoplasmic proteins called *catenins*. Each cadherin molecule can bind to either β-catenin or γ-catenin, which in turn bind α-catenin. α-Catenin links the cadherin complex to the actin cytoskeleton. Cell lines that lack α-catenin lose normal cell-cell adhesiveness, and tumor cells with mutated or downregulated α-catenin have increased invasiveness.[72]

E-cadherin is the predominant type of cadherin expressed in epithelial tissue. Alterations of E-cadherin expression and function have been observed in human cancers.[73] In addition, downregulation of E-cadherin correlates with increased invasiveness, metastasis, and poor prognosis in cancer patients. Supression of this invasive phenotype can be achieved by transfection of E-cadherin cDNA into carcinoma cells, and contrarily, invasiveness of E-cadherin gene-transfected cells can be restored by exposure of the cells to E-cadherin antibodies or an E-cadherin antisense RNA.[73] Germline mutations of the E-cadherin gene (cdh1) have been found in New Zealand Maori families with a dominantly inherited susceptibility to gastric cancer.[74] TOP

Production of Lytic Enzymes.

Transformed malignant cells in culture and human cancer cells in vivo produce a variety of lytic enzymes that degrade the ECM and allow cancer cells to invade tissues, lymphatic channels, and the vasculature. These proteases include plasminogen activator, cathepsins, and a number of matrix metalloproteases (MMPs). The MMPs are a large family of proteases that includes collagenases (MMPs1, 2, and 9) and stromelysins (MMPs 3 and 11). Collagenases have been found at elevated levels in melanoma and in cancers of the colon, breast, lung, prostate, and bladder. Usually, these elevated levels correlate with higher tumor grade and invasiveness. MMP-2 levels are significantly elevated in the serum of patients with metastatic lung cancer, and in those patients with high levels, response to chemotherapy is diminished.[75] TOP

Genetic Alterations in Cancer Cells

Suffice it to say here that cancer is essentially a genetic disease, in that all cancer cells have some alteration of gene expression. These genetic alterations include chromosomal translocations and inversions, gene deletions, gene amplifications, point mutations, and duplications or losses of whole chromosomes. Much of the information about genetic alterations in cancer has been gleaned from studies of leukemias and lymphomas because it is easier to obtain relatively pure populations of cells. Nevertheless, a significant amount of information has been obtained about genetic changes in solid tumors, in which gene deletions (e.g., loss of tumor suppressor gene function) and oncogene activation (e.g., k-*ras* mutations) are a common phenomenon.

Alterations in Chromatin Structure and Function.

Chromatin in higher organisms is organized into nucleosomes that are tuna fish can–shaped structures made up of two molecules each of the core histones H2A, H2B, H3, and H4, forming an octamer core around which close to two turns of DNA are wrapped. In a tightly wrapped conformation, DNA transcription into mRNA is inhibited. The initiation of gene transcription requires a partial unwrapping of this octamer core, which is regulated by biochemical alteration of the core histones. The mechanisms

involved in this are still only partially understood, but it involves chemical modifications that regulate the acetylation and phosphorylation states of histones. The processes of controlling chromatin structure and function are key to understanding cell differentiation and the altered gene expression that occurs in malignant transformation.

Some of the genes involved in the acetylation and deacetylation of histones have been identified.[76] The acetylation genes are of two categories: *hat1* and *hat2*. Acetylation of histone H4, for example, reduces the affinity of the histone amino terminal tail for DNA and allows a reduction of DNA wrapping around the histone octamer and a subsequent decrease in the tightness of nucleosome packaging. This makes more DNA sequences available for transcription. Deacetylation of histone H4 by deacetylases (HDAC1 and HDAC2), on the other hand, increases affinity of H4 for DNA and results in tighter packing of nucleosomes and less transcription. Mutations in yeast deacetylases have been identified that allow H3 and H4 acetylation to be maintained. This would be expected to result in constitutively unfolded regions of chromatin and increased gene transcription. Disruption of deacetylase activity that alters expression of many genes in yeast as well as mammalian cells has been observed.[76] Mutations in histone acetylases, deacetylases, and components of these complexes have significant effects in yeast cells, and similar mutations may have implications for human disease, including cancer. Recent data have shown that members of the HDAC1 and HDAC2 family of genes belong to a network of genes coordinately regulated and involved in chromatin remodeling during cell differentiation.[77]

In addition to acetylation, phosphorylation of histones is also important for chromatin structure and function.[78] A fifth histone, H1, interacts with DNA, links adjacent nucleosome cores, and further condenses chromatin structure. Phosphorylation of H1 is thought to play a role in increased gene transcription. Phosphorylation of histone H3, on the other hand, is required for proper chromosome condensation and segregation during mitosis.[78] In addition, during the immediate-early response of mammalian cells to mitogens, histone H3 is rapidly and transiently phosphorylated by a kinase called Rsk-2.[79] This suggests that chromatin remodeling is part of the cascade involved in mitogen-activated protein kinase–regulated gene expression.

A "cancer-chromatin connection" is implicated by the observations relating to the role of the tumor-suppressor gene *rb* in the regulation of the histone deacetylase HDAC1.[80] *Rb* acts as a strong transcriptional repressor by forming a complex with the transcriptional activating factor E2F and HDAC1, tethering these activities to E2F-responsive promoters, including the cyclin E promoter region. Repression of E2F-bound promoters by *rb* is released by mitogenic signals that activate cyclin-dependent kinase phosphorylation of *rb*, thereby releasing *rb* from the complex and allowing histone acetylation to occur. This increases accessibility of gene promoter sequences to transcriptional activators. Point mutations of *rb* observed in

some tumors abolish *rb*-induced repression and *rb*-associated deacetylase activity, allowing increased E2F-mediated gene expression. Viral oncoproteins can disrupt the interaction between *rb* and HDAC1. In addition, nonliganded retinoic acid receptors (RARs) have been shown to repress transcription of target genes by recruiting the histone deacetylase complex to these genes.[81] Mutant forms of RAR-$_\alpha$ result from chromosomal translocations seen in human acute promyelocytic leukemia (APL). These mutant forms prevent appropriate deacetylase activity and result in dysregulated gene activation. This dysregulation can be diminished by all-transretinoic acid, at doses that induce APL cell differentiation. These findings suggest that oncogenic alterations in RARs mediate leukemogenesis via aberrant regulation of the histone acetylation state.

DNA Methylation.

Methylation of DNA on cytosine in CpG islands is another mechanism for regulating gene expression. In general, though not always, hypermethylated DNA sequences are less expressed, and hypomethylated sequences are more expressed. CpG islands are short sequences rich in CpG dinucleotides found in the 5'- regulatory regions of about half of all human genes. Alterations in DNA methylation patterns have been observed in tumor cell lines, animal tumor models, and primary human cancers. Feinberg et al.[82] observed an average of 8 to 10% reduction in genomic 5-methylcytosine content in colon adenomas and adenocarcinomas. Interestingly, three patients with the highest 5-methylcytosine content in their normal colon appeared to have a germline predisposition to cancer (Lynch syndrome). Hypermethylation of DNA has been postulated to be involved in the loss of tumor suppressor gene function. Hypermethylation of the regulatory sequences of some of those genes, including *p15*, *p16*, E-cadherin, *vhl*, and *hmlh1*, has been observed, but whether this is a cause of tumor suppressor gene silencing is still unclear. Aberrantly methylated CpG sequences have been detected in serum and tissue of patients with colorectal, [83] non–small cell lung,[84] and liver cancers.[85]

DNA methyltransferase activity has been reported to be overexpressed in a number of human cancer cell lines and tissues, although the incidence and extent of this is still being debated.[86] So far, three DNA methyltransferases have been detected in mammalian cells,[86] and the activity of one of these, DNMT1, is three-fold higher in *fos* oncogene–transformed fibroblasts than in normal fibroblasts, and the transformed cells contain more 5-methylcytosine than normal fibroblasts.[87] Transfection of the *dnmt1* gene into fibroblasts induces transformation, whereas inhibition of *dnmt1* expression by an antisense oligonucleotide reverses *fos*-induced transformation. These results suggest that oncogene-induced malignant transformation is mediated through alterations in DNA methylation. TOP

Loss of Heterozygosity.

Deletion of genetic material is a very common event in human cancer.

Indeed, it is the most frequently observed genetic abnormality in solid tumors. These deletion events often involve loss of heterozygosity (LOH) of the expression of either the maternal or paternal alleles of a gene. If this is accompanied by mutation of the remaining allele, as is sometimes the case for a tumor suppressor gene such as *p53*, an important mechanism to regulate cell proliferation and differentiation is lost. An early observation of LOH in human cancer was by Solomon et al.,[88] who showed that about 20% of human colorectal cancers had undergone allelic loss on chromosome 5q. Vogelstein and his colleagues subsequently reported how a series of genetic alterations, including LOH of alleles at chromosomal regions 5q (*apc* gene), 17p (*p53* gene), and 18q are involved in progression of colorectal cancer.[89]

It is now recognized that LOH occurs in most, if not all, human solid tumors and may involve up to 20% of the genome. In some cancers, including lung, ovarian, and colorectal cancers, LOH is an early event and may occur at the stage of dysplasia or carcinoma in situ. The prevalence of LOH differs at different positions within the genome and is more prevalent at certain "hot spots." Frequently involved allelic loss occurs in cancer cells on chromosomes 3p, 5q, 7q, 8q, 9p, 13q, 17p, and 18q. These losses often involve regions containing tumor suppressor genes. The tumor suppressor gene functions contained in these regions include *p53*, *brca1*, *rb*, *brca2*, *apc*, *vhl*, and *p16*. LOH is detected using molecular genetic techniques such as restriction fragment length polymorphism (RFLP) or PCR. It is of interest that frequently the same genes that have undergone LOH in hereditary cancers also undergo LOH in "spontaneous" cancers. TOP

Loss of Genomic Imprinting.

Genomic imprinting is an epigenetic modification of the genome that allows only one of the maternal or paternal alleles of a gene to be expressed. So far, about 30 mammalian genes are known to be imprinted.[90] In a number of human cancers, loss of imprinting (LOI) occurs, allowing both the maternal and paternal alleles to be expressed. If this occurs for a growth factor, such as insulin-like growth factor-2 (IGF-2), cells get a double dose of a growth stimulatory signal. LOI of IGF-2 has been observed in about 45% of a series of patients with colorectal cancer.[91] Interestingly, this LOI could also be detected in patients' circulating leukocytes, suggesting that this is an alteration that precedes the onset of neoplasia and could be used as a screening test for cancer susceptibility. Somewhat paradoxically, LOI can be reversed by drugs that are DNA methyltransferase inhibitors, such as 5-aza-2-deoxycytidine, suggesting that an aberrant DNA methylation event induces LOI.[92] LOI of the igf2 gene appears to be involved in tumor progression, leading to a more invasive phenotype.[93] TOP

Telomeres and Telomerase.

Normal human cells undergo a finite number of cell divisions when grown in culture and ultimately stop dividing and undergo what is called "replicative senescence." For human cells, the number of cell divisions attained before

senescence ensues is about 50.[94] One difference between young, replicating cells and their senescent counterparts is the length of specialized "tails" at the end of chromosomes, called *telomeres*. In human cells, telomeres are made up of an average of 5,000 to 15,000 base-pair repeats containing the sequence (TTAGGG)n together with telomere-binding proteins.[95] Younger cells have the longer telomeres. Every time a cell divides, 50 to 100 base pairs are lost, and a signal is triggered to the cell to stop dividing.

Cells of higher eukaryotic organisms maintain telomere length by the activity of an enzyme complex called *telomerase*. This is a ribonucleoprotein complex that contains several proteins and RNA. The catalytic component of this complex is a reverse transcriptase, *human telomerase reverse transcriptase* (hTERT), that uses the RNA contained in the complex as a template for reverse transcription to replicate the DNA sequences in the telomere. Germ cells and pluripotent tissue stem cells have telomerase activity; however, telomerase is turned off in cells from most tissues as they differentiate. Most human cancers appear to be able to reactivate telomerase activity, thus rejuvenating their proliferative capacity.[96] This makes telomerase a hot target for both diagnostic and therapeutic approaches to cancer. There are also data indicating that restoring telomerase in human cells extends their lifespan,[97] suggesting that senescence can be overcome and perhaps providing a way to maintain human stem cells for replacement of aging or damaged tissues. TOP

DNA Repair.

DNA repair mechanisms are covered extensively in Chapter 6. It is sufficient to note here that a number of biochemical mechanisms are invoked by human cells when their DNA is damaged by internal metabolic events (e.g., oxidative stress, cytosine deamination) or exogenous factors (e.g., chemical carcinogens, irradiation). These repair mechanisms include (1) photoactivation repair for removal of UV-induced pyrimidine dimers; (2) strand-break repair, for excision and repair of a length of DNA sequence; (3) base-excision repair producing apurinic or apyrimidinic sites in DNA; (4) nucleotide excision repair; and (5) O^6-alkylguanine-DNA alkyltransferase that recognizes and removes small alkyl adducts from DNA.

DNA repair is usually very accurate, but if repair cannot occur prior to or during DNA replication, it may be error prone, potentially leading to a mutagenic and carcinogenic event. A number of inherited defects in DNA repair systems predispose individuals to getting cancer. These syndromes include xeroderma pigmentosum, ataxia telangiectasia, Fanconi's anemia, Bloom's syndrome, and Cokayne's syndrome. TOP

Alterations of Cellular Differentiation in Cancer

A cancer develops from cells that are capable of dividing. All tissues in the body contain some cells that can divide and renew themselves. A subset of the cell population in any tissue can differentiate into the functional cells of

that tissue. The normal process of cellular differentiation ultimately leads to an adult, fully differentiated, "dead-end" cell that cannot, under ordinary circumstances, divide again. These fully differentiated cells are the workhorse cells in most tissues in the body. Under circumstances that are not clearly understood, cells that have the potential to divide can be changed by interaction with carcinogenic agents into a cell type that is capable of continued proliferation and thereby is prevented from achieving the normal state of complete differentiation. The carcinogen-altered cell is said to have undergone malignant transformation. Somehow, the genes controlling cell proliferation are locked in the "on" position when they should be in the "off" position, and the genes controlling differentiation are either not expressed or are expressed only imperfectly. What we need to know to understand carcinogenesis and to develop ways of preventing or curing cancer, then, is contained in the mechanisms of normal cellular differentiation. Only by understanding these mechanisms can the manner in which cells are altered during malignant transformation be ascertained.

Differentiation is the sum of all the processes by which cells in a developing organism achieve their specific traits. By acquisition of these special traits, progeny cells are distinguishable from their parent cells and from each other. Somatic cells that share a set or a subset of structural and functional characteristics become organized into tissues in higher organisms. Indeed, cellular differentiation is the *sine qua non* of multi-cellular life.

The process of differentiation appears to be fairly permanent, in that as tissues develop, some cells retain the capacity to divide, whereas others divide and then differentiate into cells with a more restricted phenotype.[98] These latter cells are then said to be pluripotent rather than totipotent, that is, they are now committed to develop into one of the cell types peculiar to their tissue of origin. Embryologists have traditionally defined the commitment of a cell to one general pathway of differentiation rather than another as *determination*. They reserve the term *differentiation* for the final events in which a terminally differentiated cell arises from a pluripotent one. However, biochemically, this is probably an artificial distinction because the total process most likely represents a continuum of biochemical and molecular events leading from a totipotential cell to a terminally differentiated one. The final characterization of differentiation requires the identification of the particular biochemical events that lead to the uniquely specialized adult cell. By definition, the process of differentiation requires a heritable alteration in the pattern of gene readout in one of the two progeny cells arising from the same parent cell. Because all the cells in the body are derived from a single cell, the fertilized ovum, this process must entail the expression of characteristics in one progeny cell that are not expressed in the other progeny cell from the same parent cell, and this process must continue to occur throughout embryonic development to generate the wonderful diversity of cell types present in the adult organism.

Getting to Know all the Players.

The process of early development is a complicated one, and there are some

similarities and some differences among various multi-celluar organisms. The biochemical signals and genes involved, however, show a lot of evolutionary conservation. Various polypeptide growth factors have been shown to play a role in early morphogenesis.[99] For example, in early *Xenopus* development, there are a series of inductive events that involve growth factors, whose actions lead to differentiation of mesoderm at the interface between the animal and vegetal poles of the embryo. This induction is most efficiently achieved by a combination of members of the fibroblast growth factor (FGF) and transforming growth factor β (TGF-β) families of growth factors. In *Xenopus*, *Drosophila*, and developing chick limb buds the role for members of the FGF and TGF-β families of polypeptide growth factors appear to act in early development by regulating expression of *hox* genes.[99–102] For example, growth factors regulate expression of a *hox* gene called *xhox3* in *Xenopus* that is required for anterior-posterior patterning. Similar observations have been made in *Drosophila*. Since *hox* genes themselves code for transcriptional regulators that can turn genes on or off, some of which may code for growth factor–like substances, one can visualize a cascade of events in which a local concentration of growth factor turns on a *hox* gene, which, in turn, activates another growth factor that turns on another *hox* gene in a responding cell, suggesting a way that pattern formation could be transmitted from one cell region to another.

The activation of *hox* genes, however, does not clearly explain how, for example, within a given mesodermal area, different mesodermal cell types arise because *hox* genes are expressed, albeit perhaps at different times and levels, throughout the mesodermal layer. Thus, additional genes must be expressed in a carefully regulated way to lead to further "subspecialization" or differentiation events. One well-studied example of this is the expression of genes involved in the muscle differentiation pathway, for example, the myogenic genes *myo D* and myogenin.

Other important parameters of morphogenesis include the ability of like cells to cluster together and "talk to each other" and the ability of cells to produce and interact with a specific tissue type ECM. Thus, the ability to regulate cell-cell and cell-ECM (cell-substratum) interactions is also key to normal development and cellular differentiation. Two families of adhesion molecules are involved: cell-cell adhesion molecules or CAMs and cell-substratum adhesion molecules or SAMs.[102,103] CAMs produce cell-cell contact between like-minded cells that foster their interactions and cell sorting into homogeneous populations. As noted above, CAMs, or cadherins as they are also called, are large transmembrane proteins that interact through cytoplasmic connections called catenins that link cadherins to the cell cytoskeleton, thus providing an internal signaling process for CAMs that are in contact with the extracellular environment. These interactions are capable of modulating formation of actin cables in the cytoplasm and, thus, of affecting cell migration and cell surface polarity.

Thus, a number of key interactions among growth factors, *hox* genes, CAMs, SAMs, the ECM, and specific genes involved in cell lineage–specific pathways occur during early development and early differentiation. Although

mostly studied in lower organisms, all these genes have homologous counterparts in mammalian cells. TOP

Reversibility of Differentiation.

It is generally agreed that the malignant neoplastic cell is less differentiated than the normal adult cell in the organ from which the cancer originates. However, for a long time, there has been a controversy among cancer biologists on the question of whether neoplastic transformation results from de-differentiation of normal adult type cells or from the abnormal differentiation of committed, but not yet mature, stem cells in tissues. It can be concluded, however, that malignant neoplastic transformation occurs only in cells that are capable of dividing. Hence, brain tumors are not likely to arise from a mature neuron nor are leukemic cells likely to arise from a terminally differentiated polymorphonuclear leukocyte. It is more likely that the dividing, committed stem cell of a given tissue would be the most affected by oncogenic agents. However, it is difficult to define the stem cell population in some tissues. There are clearly identifiable stem cells in the bone marrow, skin, and gastrointestinal epithelium, but not in all tissues. In general, it appears that commitment to a given pathway of cellular differentiation is irreversible, but the steps of terminal differentiation may be reversible. TOP

Induction of Differentiation in Cancer Cells.

There are a number of examples of animal malignant tumors or human cancer cells in culture that can be induced to lose their malignant phenotype by treatment with certain differentiation-inducing agents. These include induction of differentiation of the Friend virus–induced murine erythroleukemia by dimethylsulfoxide (DMSO); differentiation of murine embryonal carcinoma cells by exposure to retinoic acid, cAMP analogues, hexamethylbisacetamide, or sodium butyrate; and differentiation of human acute promyelocytic (HL-60) cells in culture by a number of anticancer drugs, sodium butyrate, DMSO, vitamin D3, phorbol esters, or retinoic acid analogues.[104]

Being able to treat cancer through induction of cellular differentiation is an attractive idea because the therapy could be target cell specific and most likely be much less toxic then standard chemotherapeutic agents. The best example of this is the treatment of acute promyelocytic leukemia in patients with all-transretinoic acid. A more recent example is induction of solid tumor differentiation by the peroxisome proliferator-activated receptor-γ (PPAR-γ) ligand troglitazone in patients with liposarcoma.[105] PPAR-γ is a nuclear receptor that forms a heterodimeric complex with the retinoid X receptor (RXR). This complex binds to specific recognition sequences on DNA and, after binding ligands for either receptor, enhances transcription differentiation–inducing genes, including those for the adipocyte-specific pathway. Other PPAR-γ ligands are under development, and some of these may prove useful for treatment of sarcomas as well as other tumor types. TOP

Alterations in Signal Transduction Mechanisms

The only point to be made here is that a large number of growth factors, cytokines, hormones, and exogenous chemicals can trigger cellular responses via receptor-mediated events that foster cellular proliferation and/or differentiation. Sometimes these factors do both. The intracellular signaling pathways that accomplish this are varied and complex. Frequently, these pathways are inappropriately activated in cancer cells by either inappropriate expression of an oncogene coding for a growth factor, a growth factor receptor, or part of the intracellular signaling pathway. A key point to keep in mind is that there is significant cross-talk between these signaling pathways such that up- or down-regulation of one of them may trigger coordinate responses in another one. Thus, inhibition of one component of a signal transduction pathway may be compensated for in the cell by upregulation of another pathway. This has important therapeutic implications because a drug that blocks an early or upstream component of a given pathway may be circumvented by activation of another parallel pathway. This is seen, for example, in the development of resistance to some chemotherapeutic agents. A goal, then, is to try to target the downstream events where transduction pathways converge in their ability to stimulate gene activation events.

An example of the cross-talk among ligand-receptor triggered events is the binding of the growth factor beta platelet-derived growth factor (βPDGF) to its receptor βPDGFR.[106] This induces dimerization of the receptor, which, in turn, triggers signal transduction pathways. The βPDGF receptor becomes autophosphorylated on multiple tyrosines by activation of its receptor tyrosine kinase. This fosters binding to specific Src homology 2 domain (SH2)–containing proteins that are part of the Grb2-Sos-Ras-Raf-Mek-Erk pathway. In addition, there is cross-talk with the phosphatidyl inositol kinase (PI3K) pathway. PI3K can also stimulate Rac GTPase, which can activate JAK/STAT signaling events. Activation of the SH2 domain protein PLC-γ1 can also potentially stimulate protein kinase C (PKC) signaling pathways. Thus, cytoplasmic signaling proteins form networks of interactions rather than simple linear pathways.[106] These diverse signaling pathways, in turn, induce broadly overlapping sets of genes.[107]

GTP-binding protein (G-protein) signaling events are another ubiquitous pathway for gene activation, some of which are mediated by cyclic AMP that has protean effects on cellular processes.[108] Mutations in components of G-protein coupled pathways have been observed, some of which appear to be involved in a number of human diseases, including tumor formation.[108,109]

Alterations of other signal transduction pathways also correlate with malignant transformation. For example, cellular transforming events induced by the viral oncogene v-*fps* correlate with activation of the endogenous STAT3 signal transduction pathway.[110] TGF-β signaling is mediated via the SMAD family of transducer proteins, and somatic mutations of one of these, SMAD4, are frequently observed in pancreatic cancers and less frequently in colon, breast, and lung cancers.[111] Functionally disruptive mutations of

SMAD2 have been observed in colorectal and lung cancers.

These observations increase the long list of signal transduction components that are known to be altered in cancer, such as Ras, Myc, Src, and Erb B.[108] Thus, it is clear that disruption of signal transduction pathways is a commonly observed event in human cancer and provides a target for therapeutic intervention.

It should also be noted that the use of DNA microarray technology is providing a way to trace what happens to multiple pathways when cells are altered by external stimuli or malignant transformational events. This has fostered the new field of "pathway biology" in which we are learning that stimulation of a cell (e.g., by a growth factor) or damage to a cell (e.g., by oxidative stress) up- or downregulates the expression of a wide variety of genes that code for proteins in multiple pathways that, heretofore, we had no idea were linked.

Phosphorylation/Dephosphorylation Events.

As noted above, many signal transduction events involve phosphorylation steps. These include (1) receptors coupled to tyrosine kinase activity; (2) receptors coupled to guanine nucleotide-binding proteins, which, in turn, may activate or inhibit adenylate cyclase, activate phosphoinositide hydrolysis leading to protein kinase C activation and intracellular Ca^{++} release, or modulate cell membrane ion channels; and (3) intracellular receptors, such as those for steroid hormones, thyroid hormone, and retinoic acid, all of which have DNA-binding domains as well as ligand-binding domains and can interact directly with DNA to modulate gene transcription. All these receptor-mediated signal transduction mechanisms are potential sites for upregulation or deregulation in cancer cells, for example, by oncogene activation or overexpression or by tumor suppressor gene inactivation.

Tyrosine Kinases.

The tyrosine kinase–coupled receptors mentioned above are one potential target for carcinogenic alteration. Activation of these receptors can lead to phosphorylation of a number of key substrates. Many growth factor receptors mediate their cellular effects by intrinsic tyrosine kinase activity, which, in turn, may phosphorylate other substrates involved in mitogenesis. A number of transforming oncogene products have growth factor or growth factor receptor–like activities that work via a tyrosine kinase–activating mechanism. For example, the v-*src* gene product is itself a cell membrane associated tyrosine kinase. The v-*sis* oncogene product is virtually homologous to the B chain of PDGF. The v-*erb* product is a truncated form of the epidermal growth factor (EGF) receptor. The *fms* gene product is analogous to the receptor for CSF-1. The *met* and *trk* proto-oncogene products turn out to be receptors for hepatocyte growth factor (HGF) and nerve growth factor (NGF), respectively.

Some of the key substrates for receptor-tyrosine kinase coupled activity include (1) phospholipase C (PLC_γ), which, in turn, activates phosphatidyl inositol hydrolysis, releasing the second messengers diacylglycerol (DAG) and inositol triphosphate (INSP3) that activate protein kinase C (PKC) and mobilize intracellular calcium release (a number of tumor promoters also activate PKC); (2) the GTPase activating protein GAP that modulates *ras* proto-oncogene protein function; (3) *src*-like tyrosine kinases; (4) PI-3 kinase that associates with and may modulate the transforming activity of polyoma middle T antigen and the v-*src* and v-*abl* gene products; and, (5) the *raf* proto-oncogene product that is itself a serine/threonine protein kinase.

Thus, activation of protein kinases is a key mechanism in regulating signals for cell proliferation. The substrates of these kinases include transcription regulatory factors, such as those linked to mitogenic signaling pathways, for example, proteins encoded by the *jun*, *fos*, *myc*, *myb*, *rel*, and *ets* proto-oncogenes. TOP

Protein Phosphatases.

Although it has been known for a long time that protein phosphatases play a regulatory role in certain cellular metabolic functions, for example, in the activation-inactivation steps for glycogen synthase and phosphorylase, it has only recently been demonstrated that phosphatases play a role in the activity of various receptors and in the function of certain cell cycle regulating genes.[108] For example, expression of a truncated, abnormal protein tyrosine phosphatase in BHK cells produces multi-nucleated cells, possibly by dephosphorylating the cyclin-dependent kinase $p34^{cdc2}$. Activation of $p34^{cdc2}$ requires dephosphorylation of a tyrosine residue, and this activation drives the cell from the G2 into the M-phase. The truncated phosphatase apparently interferes with the normal synchrony between nuclear formation and cell division.

Protein tyrosine phosphatases (PTPases), it is now known, are a diverse family of enzymes that exist in cell membranes. Some of them are associated with receptors that have tyrosine kinase activity. Phosphatases are also in other intracellular locations. The aberrant phosphorylation state of tyrosine in certain key proteins, such as c-Src or c-Raf, that can lead to cellular transformation could theoretically come about due to deregulation of a protein kinase or underexpression of a protein phosphatase. For example, cells treated with vanidate, a PTPase inhibitor, have increased protein phosphotyrosine levels and a transformed phenotype.[112] Further evidence that PTPases are involved in cancer is the observation that receptor-linked PTPase $_\gamma$ (one of the PTPase isozymes) is located on chromosome 3, which has a deletion in renal cell and lung carcinomas, suggesting that the PTPase $_\gamma$ gene may act as a tumor suppressor gene. Thus, one could predict that a high level of expression of specific PTPases may be able to reverse the malignant phenotype, and one can think of strategies, then, to transfect these genes into tumor cells or deliver inducers of the enzymes to tumor cells.

A protein tyrosine phosphatase called PTEN has been found to be mutated in human brain, breast, and prostate cancers.[113] This was discovered by mapping homozygous deletions on human carcinoma 10q23 that occur at high frequency in human cancers. Mutations of the pten gene were detected in 17% of primary glioblastomas as well as in human-derived cancer cell lines and xenografts of glioblastoma (31%), prostate cancer (100%), and breast cancer (6%). PTEN is a protein tyrosine phosphatase that dephosphorylates PIP3 in the phosphatidyl inositol pathway. Loss of PTEN activity increases PIP3 phosphorylation and leads to cellular transformation. Thus, PTEN is considered to have tumor suppressor function, and this protein and its substrates are potential targets for new therapeutic agents. TOP

Alterations of Cell Cycle Regulation and Apoptosis

Cell Cycle Regulation.

Cell cycle check points occur at key transitions in the cell cycle and provide go/no go decision points that determine whether a cell progresses to the next cell cycle phase or not. The biochemical mechanisms involved in these check-point controls have now been identified. Most of what we know about cell cycle regulation originally came from lower organisms, including yeast.[114] One of the first genes to be identified as an important cell cycle regulator in yeast is *cdc2/cdc28*. Activation of this gene requires association with a regulatory subunit called cyclin A. It is now known that sequential activation and inactivation of cyclin-dependent kinases (cdks) is the primary means of cell cycle regulation. Thus, this is another example of the importance of phosphorylation/dephosphorylation in the biochemistry of cell regulation.

The role of various cdks, cyclins, and other gene products in regulating check points at G1 to S, G2 to M, and mitotic spindle segregation have been described in detail elsewhere.[114–116] Alterations of one or more of these check-point controls occur in most, if not all, human cancers at some stage in their progression to invasive cancer. Examples of some of these alterations are given below.

Alteration of the G1/S check point occurs in many human cancers. Cyclin D1 gene amplification occurs in a subset of breast, esophageal, bladder, lung, and squamous cell carcinomas. Cyclins D2 and D3 are overexpressed in some colorectal carcinomas. In addition, the cyclin D associated kinases cdk4 and cdk6 are overexpressed or mutated in some cancers. Mutations or deletions in the cdk4 and cdk6 inhibitor INK4 have been observed in familial melanomas, and in biliary tract, esophageal, pancreatic, head and neck, non–small cell lung, and ovarian carcinomas. Inactivating mutations of cdk4 inhibitory modulators p15, p16, and p18 have been observed in a wide variety of human cancers. Cyclin E is also amplified and overexpressed in some breast and colon carcinomas and leukemias.

A key player in the G1/S check-point system is the retinoblastoma gene *rb*.

Phosphorylation of *rb* by cyclin D-dependent kinase releases *rb* from the transcriptional regulator E2F and activates E2F function. Inactivation of *rb* by genetic alterations occurs in retinoblastoma and is also observed in other human cancers, for example, small cell lung carcinomas and osteogenic sarcomas.

The *p53* gene product is an important cell cycle check-point regulator at both the G1/S and G2/M check points but does not appear to be important at the mitotic spindle check point because gene knockout of *p53* does not alter mitosis. The *p53* tumor suppressor gene is the most frequently mutated gene in human cancer, indicating its important role in conservation of normal cell cycle progression. One of *p53*'s essential roles is to arrest cells in G1 after genotoxic damage, to allow for DNA repair prior to DNA replication and cell division. In response to massive DNA damage, *p53* triggers the apoptotic cell death pathway. Tumor cells lacking normal *p53* function do not arrest in G1 and are more likely to progress into S or G2/M and die, although different cells and different drugs appear to have different requirements for this cell killing effect.[117]

The spindle assembly check-point machinery involves genes called *bub* (budding uninhibited by benomyl) and *mad* (mitotic arrest-deficient).[116] There are three *bub* genes and three *mad* genes involved in the formation of this check-point complex. A protein kinase called Mps1 also functions in this check-point function. The chromosomal instability, leading to aneuploidy in many human cancers, appears to be due to defective control of the spindle assembly check point. Mutant alleles of the human *bub1* gene have been observed in colorectal tumors displaying aneuploidy. Mutations in these spindle check-point genes may also result in increased sensitivity to drugs that affect microtubule function because drug-treated cancer cells do not undergo mitotic arrest and go on to die. TOP

Apoptosis.

Apoptosis (sometimes called programed cell death) is a cell suicide mechanism that enables multi-cellular organisms to regulate cell number in tissues and to eliminate unneeded or aging cells as an organism develops. The biochemistry of apoptosis has been well studied in recent years, and the mechanisms are now reasonably well understood.[118–120] The enzymatic machinery for this was first discovered in the nematode *C. elegans*, and later the homologues of these genes and their products were identified in mammalian cells, including human cells. The apoptosis pathway involves a series of positive and negative regulators of proteases called *caspases*, which cleave substrates, such as poly (ADP-ribose) polymerase, actin, fodrin, and lamin. In addition, apoptosis is accompanied by the intranucleosomal degradation of chromosomal DNA, producing the typical DNA ladder seen for chromatin isolated from cells undergoing apoptosis. The endonuclease responsible for this effect has now been identified.[121,122]

A number of "death receptors" have also been identified.[118] Death receptors

are cell surface receptors that transmit apoptotic signals initiated by death ligands. The death receptors sense signals that tell the cell that it is in an uncompromising environment and needs to die. These receptors can activate the death caspases within seconds of ligand binding and induce apoptosis within hours. Death receptors belong to the tumor necrosis factor (TNF) receptor gene superfamily and have the typical cystine-rich extracellular domains and an additional cytoplasmic sequence termed the *death domain.* The best characterized death receptors are CD95 (also called Fas or Apo1) and TNF receptor TNFR1 (also called p55 or CD120a).

The importance of the apoptotic pathway in cancer progression is seen when there are mutations that alter the ability of the cell to undergo apoptosis and allow transformed cells to keep proliferating rather than dying. Such genetic alterations include the translocation of the *bcl-2* gene in lymphomas that prevents apoptosis and promotes resistance to cytotoxic drugs. Other genes involved as players on the apoptosis stage include c-*myc*, *p53*, c-*fos*, and the gene for interleukin-1β-converting enzyme (ICE). Various oncogene products can suppress apoptosis. These include adenovirus protein E1b, *ras*, and v-*abl*.

Apoptosis occurs in most, if not all, solid cancers. Ischemia, infiltration of cytotoxic lymphocytes, and release of TNF may all play a role in this. It would be therapeutically advantageous to tip the balance in favor of apoptosis over mitosis in tumors, if that could be done. It is clear that a number of anticancer drugs induce apoptosis in cancer cells. The problem is that they usually do this in normal proliferating cells as well. Therefore, the goal should be to manipulate selectively the genes involved in inducing apoptosis in tumor cells. Understanding how those genes work may go a long way to achieving this goal. ↑ TOP

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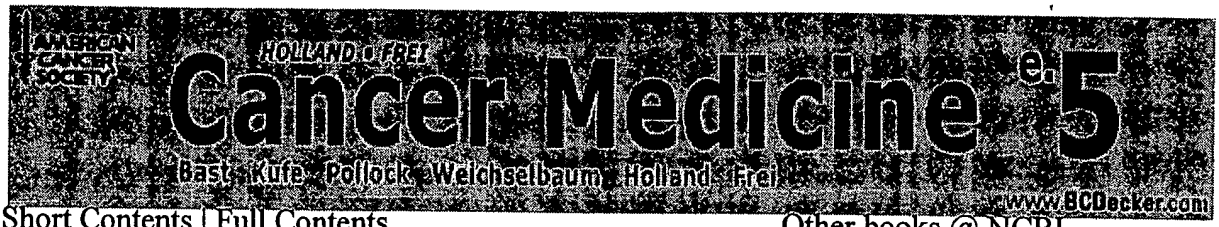

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Table 7.3. Properties of Transformed Malignant Cells Growing in Cell Culture and/or in Vivo

A. In vitro alterations

1. Cytologic changes resembling those of cancer cells in vivo, including increased cytoplasmic basophila, increased numer and size of nuclei, increased nucleus: cytoplasmic ratio, and formation of clusters and cords of cells.

2. Alteration in growth characteristics:

a. "Immortality" of transformed cells in culture. Transformed malignant cells become "immortal" in that they can be passaged in culture indefinitely.

b. Decreased density-dependent inhibition of growth or loss of "contact inhibition." Transformed cells frequently grow to a higher density than their normal counterparts, and they may "pile up" in culture rather than stop growing when they make contact.

c. Decreased serum requirement. Transformed cells usually require lower concentrations of serum or growth factors to replicate in culture than nontransformed cells require.

d. Loss of anchorage dependence and acquisition of ability to grow in soft agar. Transformed cells may lose their requirement to grow attached to surfaces and can grow as free colonies in a semisolid medium.

e. Loss of cell cycle control. Transformed cells fail to stop at cell cycle check points in the cell cycle when they are subject to metabolic restriction of growth.

f. Resistance to apoptosis (programed cell death).

3. Changes in cell membrane structure and function, including increased agglutinability by plant lectins; alteration in composition of cell surface glycoproteins, proteoglycans, glycolipids, and mucins; appearance of tumor-associated antigens; and increased uptake of amino acids, hexoses, and nucleosides.

4. Loss of cell-cell and cell-extracellular matrix interactions that foster cell differentiation.

5. Loss of response to differentiation-inducing agents and altered cellular receptors for these agents.

6. Altered signal transduction mechanisms, including constitutive rather than

regulated function of growth factor receptors, phosphorylation cascades, and dephosphorylation mechanisms.

7. Ability to produce tumors in experimental animals. This is the sine qua non that defines malignant transformation in vitro. If the cells believed to be transformed do not produce tumors in appropriate animal hosts, they cannot be defined as "malignant." However, failure to grow in an animal model does not rule out the fact that they may be tumorigenic in a different type of animal (e.g., syngenic vs. allogeneic).

B. In vivo alterations

1. Increased expression of oncogene proteins due to chromosomal translocation, amplification, or mutation.

2. Loss of tumor suppressor gene protein products due to deletion or mutation.

3. Alterations in DNA methylation patterns.

4. Genetic imprinting errors that lead to overproduction of growth processing substances, e.g., IGF-2.

5. Increased or unregulated production of growth factors, e.g., TGF-α, tumor angiogenesis factors, PDGF, hematopoietic growth factors (e.g., CSFs, interleukins).

6. Genetic instability leading to progressive loss of regulated cell proliferation, increased invasiveness, and increased metastatic potential. "Mutator" genes may be involved in this effect.

7. Alteration in enzyme patterns. Malignant cells have increased levels of enzymes involved in nucleic acid synthesis and produce higher levels of lytic enzymes, e.g., proteases, collagenases, glycosidases.

8. Production of oncodevelopmental gene products. Many cancers produce increased amounts of oncofetal antigens (e.g., carcinoembryonic antigen), placental hormones (e.g., human chorionic gonadotropin), or placental-fetal type isoenzymes (e.g., placental alkaline phosphatase).

9. Ability to avoid the host's antitumor immune response.

Adapted from Ruddon.[22] IGF = insulin-like growth factor; TGF-α = transforming growth factor = a; PDGF = platelet-derived growth factor; CSF = colony stimulating factor.

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